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Previews

Modifying Mitochondrial tRNAs: Delivering What the Cell Needs

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Protein synthesis is critically dependent on transfer (t)-RNAs, but the factors regulating tRNA function are poorly understood. In this issue of *Cell Metabolism*, Wei et al. (2015) show that Cdk5 regulatory subunit-associated protein-like-1 synchronizes mitochondrial and cytosolic translation in response to external stress, providing key insight into the pathogenesis of a common inherited mitochondrial disease.

Functional tRNA molecules provide the direct link between the DNA and protein, enabling the coordinated recruitment of amino acids based on the DNA transcript that determines the peptide secondary sequence. All tRNA molecules require a wide variety of posttranscriptionally modified nucleosides which stabilize tRNA structure, enable efficient interaction with the ribosome, and fine-tune the translation machinery (Machnicka et al., 2015). Several distinct enzymes have been shown to posttranscriptionally modify cytosolic tRNAs at over 100 different positions (Machnicka et al., 2013), and in the mitochondrion, 15 species of modified nucleosides have been described modifying 118 tRNA sites (Suzuki and Suzuki, 2014). The location and type of modification vary greatly between different tRNA molecules, organisms, and tissues. Physiological environment and growth conditions influence the RNA modification pattern, raising the possibility that tRNA modification may be important in synchronizing translation to the needs of the organism at that particular time (Machnicka et al., 2013). However, the exact role of these modifications is largely unknown. It is also intriguing that cytoplasmic and mitochondrial translation share several key components. For example, a regulatory role of aminoacyl-tRNA synthetases has been proposed for both cytosolic and mitochondrial translation, and two aminoacyl tRNA synthetases (GARS, KARS) are present in both the cytoplasm and mitochondria.

In this issue of *Cell Metabolism*, Wei et al. (2015) suggest that tRNA modification synchronizes protein translation within cytosolic and mitochondrial compartments, provide additional experimental evidence that mitochondrial (mt)-tRNAs modification has a role in stress adaptation, and finally show how this may contribute to the pathomechanism of mitochondrial diseases.

The disruption of intramitochondrial protein synthesis leads to biochemical defects affecting multiple subunits of the mitochondrial respiratory chain, causing severe human diseases. Many of these patients have a primary pathogenic mutation of mitochondrial DNA (mtDNA) which directly affects the mitochondrial (mt)-tRNA or mt-RNA genes, but approximately one-third of patients have a presumed nuclear genetic disorder. Defining the molecular basis of this group has been particularly challenging, but recent advances in massively parallel sequencing have revealed several new disease genes, and unraveled the mechanisms of both intramitochondrial and cytoplasmic translation machinery—and particularly the role of tRNA modification (Figure 1).

Myopathy, lactic acidosis and sideroblastic anemia (MLASA) has been associated with mutations in pseudouridylate synthase 1 (*PUS1*), an enzyme located in both nucleus and mitochondria. PUS1 converts uridine into pseudouridine in several cytosolic and mitochondrial tRNA positions, thereby increasing the efficiency of protein synthesis in both compartments (Fernandez-Vizarra et al., 2007). Mutations in the methionyl-tRNA formyltransferase (*MTFMT*) gene, responsible for the N-formylation of the initiator $tRNA^{Met}$ in mitochondria, result in Leigh syndrome. Unlike all other translation systems, mitochondria use a single $tRNA^{Met}$ for both initiation and elongation. A portion of Met-$tRNA^{Met}$ is formylated for initiation, whereas the remainder is used for elongation (Boczonadi and Horvath, 2014). Autosomal recessive mutations in the tRNA 5-methylaminomethyl-2-thiouridylate methyltransferase (TRMU) enzyme have been reported in reversible infantile liver disease. TRMU is responsible for the thiouridylation of the uridine at the first anticodon position (U34, wobble position), only present in the anticodon of three mt-tRNAs (Glu, Lys, and Gln). The 2-thio group is required for the efficient codon recognition, and interestingly, a modifying role of dietary cysteine intake has been suggested in the manifestation of the disease (Boczonadi and Horvath, 2014). Deficiency of the mitochondrial translation optimization factor 1 (MTO1) enzyme that catalyzes the 5-carboxymethylamino-methylation of the same U34 at the wobble position in mt-$tRNA^{Glu}$, mt-$tRNA^{Gln}$ and mt-$tRNA^{Lys}$ was reported in patients with infantile hypertrophic cardiomyopathy (Boczonadi and Horvath, 2014). Furthermore, a tissue-specific regulatory role of MTO1 has been recently suggested in fine-tuning mitochondrial translation and balancing mitochondrial and cellular secondary stress responses (Tischner et al., 2014). Mutations in *TRIT1* encoding a human tRNA isopentenyltransferase, which is responsible for i6A37 modification of the anticodon loops of a small subset of cytosolic and mitochondrial tRNAs, were identified in a patient with severe epileptic encephalopathy, diabetes mellitus. Here the abnormal mitochondrial protein synthesis led to a combined defect of mitochondrial respiratory chain complexes which were responsible for the human disease (Yarham et al.,

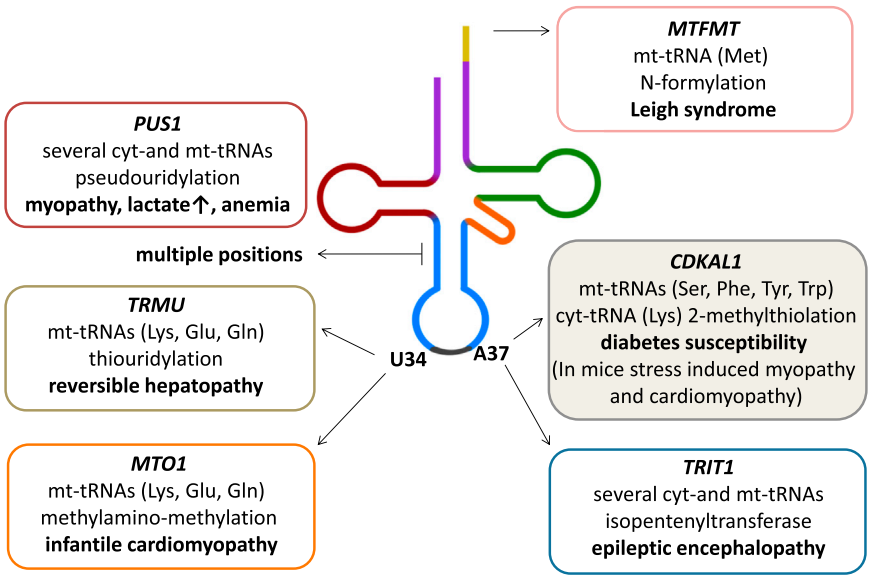

Figure 1. Human Diseases Caused by Abnormal Modification of mt-tRNAs
The figure illustrates the variety of clinical presentations caused by abnormal mt-tRNA modification due to mutations in nuclear genes.

2014). These data demonstrate that deficiencies of i6A37 tRNA modification should be considered a potential mechanism of human disease caused by both nuclear gene and mtDNA mutations. Altered tRNA modification was also documented to contribute to the pathomechanism of two common pathogenic mt-tRNA mutations (m.3243A > G, which causes mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes [MELAS], and m.8344A > G, which causes myoclonic epilepsy with ragged-red fibers [MERRF]) (Suzuki and Suzuki, 2014), and in m.3243A>G cybrids, the expression of mt-tRNA-modifying enzymes was modulated by microRNA-9/9*.

A novel role of the cell-cycle regulation enzyme cyclin-dependent protein kinase 5 (CDK5) was recently shown to regulate the synthesis of both mitochondrial and cytoplasmic proteins (Reiter et al., 2012). An aberrant regulation of CDK5 leads to the development of various neurodegenerative diseases including Alzheimer's disease. Recently, CDK5RAP1 was shown to inhibit the active CDK5 kinase through a complex modification step (N6-isopentenyladenosine [i(6)A] into 2-methylthio-N6-isopentenyladenosine [ms(2)i(6)A]). This conversion affected both mitochondrial and cytosolic tRNAs, providing a link between kinase-based protein modification and RNA modification (Reiter et al., 2012). In this issue, Wei et al. (2015) advance our understanding of this important pathway by showing that another regulatory unit—Cdk5 regulatory subunit-associated protein-like-1 (CDKAL1)—is responsible for 2-methylthio (ms^2) modification of cytosolic $tRNA^{Lys(UUU)}$, and the mitochondrial tRNAs, $mt\text{-}tRNA^{Ser(UCN)}$, $mt\text{-}tRNA^{Phe}$, $mt\text{-}tRNA^{Tyr}$, and $mt\text{-}tRNA^{Trp}$. Homozygous Cdkal1 knockout mice developed a respiratory chain deficiency due to abnormal intramitochondrial protein synthesis, and under stress conditions (ketogenic diet) these mice developed myopathy and cardiomyopathy.

More importantly, Wei et al. (2015) showed that ms^2 modifications of mt-tRNAs are sensitive to oxidative stress, and are reduced in peripheral blood cells of MELAS patients carrying the m.3243A > G mutation. Patients with the m.3243A > G mtDNA mutation invariably harbor a mixture of mutated and wild-type mtDNA (heteroplasmy) in varying proportions. Wei et al. (2015) harnessed this complexity to show a correlation between the percentage level of the m.3243A > G mutation and the degree of ms^2 modification. Given that the level of heteroplasmy correlates with the clinical features, their findings provide strong evidence that the mt-tRNA modifications contribute to the pathogenesis of this common mitochondrial disorder.

Taken together, these findings raise the possibility that similar tRNA modifications play a crucial role in regulating cellular energy delivery in response to local needs, and also implicate dysfunctional mt-tRNA modifications in other mtDNA tRNA disorders. This is exciting, because at present the large group of inherited disorders of intramitochondrial protein synthesis have no treatment. Unveiling this new mechanism presents a tractable target that can be manipulated by external factors, perhaps leading us to the treatment "holy grail."

REFERENCES

Boczonadi, V., and Horvath, R. (2014). Int. J. Biochem. Cell Biol. *48*, 77–84.

Fernandez-Vizarra, E., Berardinelli, A., Valente, L., Tiranti, V., and Zeviani, M. (2007). J. Med. Genet. *44*, 173–180.

Machnicka, M.A., Milanowska, K., Osman Oglou, O., Purta, E., Kurkowska, M., Olchowik, A., Januszewski, W., Kalinowski, S., Dunin-Horkawicz, S., Rother, K.M., et al. (2013). Nucleic Acids Res. *41* (Database issue), D262–D267.

Machnicka, M.A., Olchowik, A., Grosjean, H., and Bujnicki, J.M. (2015). Published online January 22, 2015. http://dx.doi.org/10.4161/15476286.2014.992273.

Reiter, V., Matschkal, D.M., Wagner, M., Globisch, D., Kneuttinger, A.C., Müller, M., and Carell, T. (2012). Nucleic Acids Res. *40*, 6235–6240.

Suzuki, T., and Suzuki, T. (2014). Nucleic Acids Res. *42*, 7346–7357.

Tischner, C., Hofer, A., Wulff, V., Stepek, J., Dumitru, I., Becker, L., Haack, T., Kremer, L., Datta, A.N., Sperl, W., et al. (2014). Hum. Mol. Genet. *2014*, 30.

Wei, F.Y., Zhou, B., Suzuki, T., Miyata, K., Ujihara, Y., Horiguchi, H., Takahashi, N., Xie, P., Michiue, H., Fujimura, A., et al. (2015). Cell Metab. *21*, this issue, 428–442.

Yarham, J.W., Lamichhane, T.N., Pyle, A., Mattijssen, S., Baruffini, E., Bruni, F., Donnini, C., Vassilev, A., He, L., Blakely, E.L., et al. (2014). PLoS Genet. *10*, e1004424.